Stimulation of *Nicotiana tabacum* pollen tube growth by γ-irradiation

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Accepted 11 February 1989

Irradiation of pollen grains from *Nicotiana tabacum* with ^{60}Co-γ-irradiation in the dose range of 1–6 Gy stimulates growth of the pollen tube up to 50% above control level. Further increase of the irradiation dose to 12 Gy reduces pollen growth approximately to control level. Pollen tube growth was measured photometrically by determination of the turbidity of a suspension of the fragmented pollen tubes. The sensitivity of *Nicotiana tabacum* pollen to γ-irradiation is higher than that of pollen from pine and Douglas-fir. The biological and environmental implications are discussed.

Bestraling van *Nicotiana tabacum*-stuifmeelkorrels met ^{60}Co-gammastrale in die dosis-reikwydte van 1–6 Gy, stimuleer die groei van die stuifmeelbuis op tot 50% bokant die kontrole-vlak. Verdere vermeerdering van die bestralingsdosis tot 12 Gy, verminder die stuifmeel-groei ongeveer tot die kontrole-vlak. Die groei van die stuifmeelbuis was fotometries gemeet deur bepaling van die troebelheid van 'n suspensie van die gefragmenteerde stuifmeelbuise. Die sensitiwiteit van *Nicotiana tabacum*-stuifmeel tot gammabestraling is hoër as die stuifmeel van denne en Douglas-denne. Die biologiese en omgewingsimplikasies word bespreek.

Keywords: γ-irradiation, pollen tube growth, turbidity assay

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Introduction

The germination of plant pollen and the growth of the pollen tube depend on a number of biological and environmental factors, amongst which season, collection methods, temperature and medium composition rank as the most critical [see Stanley & Linskens (1974) for review]. It is also well established that toxic substances and calcium antagonists influence germination and inhibit the growth of the pollen tube (Gentile *et al.* 1973; Gentile *et al.* 1978; Pfahler 1981; Picton & Steer 1985; DuBay & Murdy 1983; Scholz *et al.* 1985). The growth rate of the pollen tube has recently been used as a sensitive indicator of pesticides and environmental pollutants and forms the basis of various bioassays (Kappler & Kristen 1987, 1988; Meyberg *et al.* 1987).

We have exposed pollen grains of *Nicotiana tabacum* to ^{60}Co-γ-irradiation in order to investigate the influence of irradiation on the growth of the pollen tube.

Methods

Pollen collection

Unopened flowers from tobacco plants (*Nicotiana tabacum*, elsoma), grown in open fields at Elsenburg Agricultural College, Stellenbosch, were collected in the early morning during January and February. The flowers were spread out on a table and allowed to dry out over 2 days at room temperature. The pollen was shaken into preweighed plastic beakers, and the yield determined, before aliquoting into scintillation vials and storage at 4°C.

Pollen culture

Pollen was evenly suspended, using a Potter-Eljeham Teflon-glass homogenizer, at a concentration of 1 mg pollen per ml of aqueous culture medium (10% w/v sucrose, 0.01% w/v boric acid, 10 mM 2(N-morpholino)-ethanesulfonic acid (MES) adjusted to pH 5.6 with potassium hydroxide. Germination was initiated by the addition of 10 µl of a 300-µM solution of calcium nitrate to the cultures of pollen (1 ml) in glass scintillation vials. The capped vials were incubated for 2 h at 25°C in the dark on an orbital shaker, and then exposed to various doses of radiation from a ^{60}Co-γ source, at a dose rate of 2.8 Gy min^{-1}. Controls representing 100% pollen tube growth were not irradiated. Pollen grains fixed in the culture medium by the addition of formaldehyde (final concentration 10%), were used to establish a zero growth control. Following irradiation, the cultures were returned to the incubator for a further 18 h.

Determination of tube growth

The germinated pollen, and the associated pollen tubes, were isolated from the ungerminated grains by passive filtration through nylon gauze (80-µm mesh) and resuspended in 9 ml water. After centrifugation (1 000 g for 10 min), 7 ml of the supernatant was removed, and the pelleted pollen grains and tubes homogenized. The turbidity of the suspension was measured at a wavelength of 500 nm. The mean photometric value of the zero growth controls was subtracted from the value measured in the other vials. Tube wall growth in the irradiated cultures was expressed as a percentage of the non-irradiated (100%) growth controls.

Results and Discussion

The yield of pollen grains was approximately 1 g per 1 000 tobacco flowers. The average germination rate was

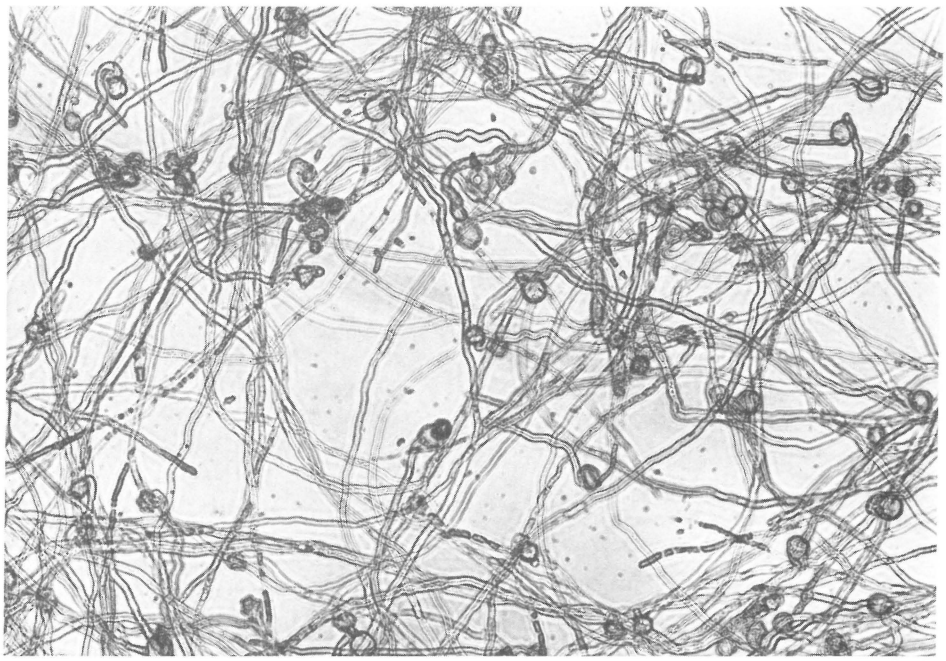

Figure 1 *Nicotiana tabacum* pollen 18 h post-germination — magnification × 60. Pollen tubes were approx. 2 mm in length and similar to the growth of *Nicotiana sylvestris* pollen germinated *in vitro* (Kandasamy & Kristen 1987).

found to be 30%. A higher and more consistent germination rate can be obtained from plants cultivated under controlled conditions. This is in line with the observation that intact anthers obtained from flowers which were still closed or just beginning to open produced pollen of better viability than pollen collected from open flowers where the anthers had started to release the pollen grains. The conditions chosen for germination supported vigorous tube growth (Figure 1) and turbidity measurements indicated good reproducibility between batches.

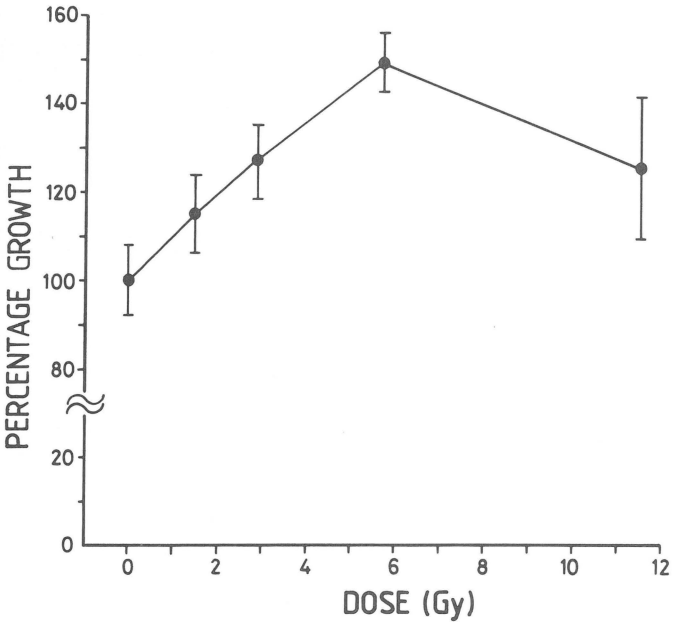

Figure 2 Growth of pollen tubes of *Nicotiana tabacum* in response to exposure of pollen grains to ^{60}Co-γ-irradiation. Germinated pollen and pollen tubes were separated from the ungerminated pollen grains and total biomass was determined spectrophotometrically at 500 nm.

Exposure of ungerminated pollen grains to ^{60}Co-γ-irradiation was found to result in a biphasic dose response. At doses of 1–6 Gy, pollen tube growth was distinctly stimulated, reaching 50% above control at a dose of 6 Gy. Doses in excess of 6 Gy up to a level of 12 Gy reduced pollen tube growth approximately to control level (Figure 2). Growth measurement by determination of turbidity of a suspension of the fragmented tubes was complicated by the difficulty of reducing particle size sufficiently to obtain optically stable suspensions. This is reflected by the large error bars associated with the individual measurements (4 replicates). A different and promising approach to the determination of tube biomass is that of Alcian Blue binding. This stain binds to the tube wall and cytoplasmic polysaccharides and can be extracted and read in a spectrophotometer (Kappler & Kristen 1988).

That low doses of X- or γ-irradiation may act as a growth stimulant has been observed in pea seedlings (*Pisum sativum*) by Bagi *et al.* (1988) and in bean roots (*Vicia faba*) by Gray & Scholes (1951). Observations of Pfahler (1981) on maize pollen (*Zea mays*) indicate that UV irradiation (280–320 μm) produces no change in germination but a sharp inhibition of pollen tube growth.

The subject of growth stimulation of plant pollen by ionizing radiation has been somewhat controversial (Brewbaker & Emery 1962) but elaborate and statistically well-designed work on pine pollen (*Pinus sylvestris*) by Zelles & Seibold (1976) and on Douglas-fir pollen (*Pseudo tsuoa menzii*) by van der Donk *et al.* (1978) and Livingston & Stettler (1973) have demonstrated enhanced pollen tube growth in response to 10 and 2 500 Gy respectively. The effect was detected by length

measurement and found to be dose-rate dependent (Zelles & Seibold 1976). The influence of ionizing radiation manifests itself at the vegetative and reproductive level. High doses of ionizing irradiation in the range of 100 Gy and above are known to fragment and eliminate certain parts of the parental genome. This effect has found application in plant breeding to select for desirable characteristics as exemplified in work on tomato (Zamir 1983), tobacco (Jinks *et al.* 1981), barley (Powell *et al.* 1983) and citrus (de Lange & Vincent 1988). The effect on vegetative growth of the pollen tube appears as yet unexploited. The mechanism of radiation-induced growth stimulation is still poorly understood. Van der Donk *et al.* (1978) observed that doses up to 2 500 Gy enhance the processing of paternal RNA and stimulate polysome activity in Douglas-fir pollen. It therefore seems unlikely that irradiation acts at the level of transcription.

The possibility of over-compensated repair has been considered but careful experiments by Zelles *et al.* (1977) show that selective irradiation of the cytoplasm with UV light produces a greater growth stimulation than irradiation of the active vegetative nucleus. It therefore seems that both DNA and RNA feature as critical targets but that processing of RNA and not repair of DNA may be the ultimate stimulus.

The growth stimulation of *Nicotiana tabacum* pollen tubes by doses of 2–6 Gy contrasts with pine pollen which requires 20–100 Gy (Zelles & Seibold 1976) and with Douglas-fir pollen which requires up to 2 500 Gy (van der Donk *et al.* 1978). This differential sensitivity of species could form the basis of a range of environmental pollution indicators.

Acknowledgements

This work was supported by the Medical Research Council of S.A. and the National Cancer Association. We thank Professors U. Kristen, N. van Schyf and Dr H. de Lange for suggestions and comments and Mr A. Serafin for assistance.

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